

whether a small movement of the slit caused a greater difference in hue, with or without white, the above results were confirmed, *i.e.*, in the case of yellow and red, no increase due to the white was observable, and in the case of the green a small addition of white seemed to make the difference in hue very slightly more pronounced.

The above observations seem to indicate that difference in our power of appreciating differences in hue, according as we are comparing two monochromatic patches, or a single patch in which the hue changes gradually from one side to the other, is not due to admixture of white light.

On the Direct Guaiacum Reaction given by Plant Extracts.

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(Communicated by W. Bateson, F.R.S. Received April 25,—Read May 18, 1911.)

In the literature dealing with oxidising enzymes considerable attention has been drawn to the fact that the juices of some plants blue guaiacum tincture directly (direct action), whereas the juices of other plants only bring about the blueing on addition of hydrogen peroxide (indirect action).

As an explanation of this phenomenon Chodat and Bach formulated a hypothesis which has, in part, been generally accepted. These authors maintain that direct blueing of guaiacum is brought about through the activity of a system consisting of oxygenase, peroxide, and peroxidase. The peroxidase is an enzyme capable only of transferring oxygen from the peroxide to the guaiacum. The peroxide, after reduction, is again re-oxidised by a second enzyme, the oxygenase. The juices of such plants as give a direct action contain, according to Chodat and Bach, all three components of the system. In others, the peroxidase alone is present, and hence the guaiacum cannot be oxidised until a peroxide, such as hydrogen peroxide, is artificially supplied.

In a recent paper Moore and Whitley* have cast considerable doubt upon the existence of any such enzyme as an oxygenase and give experimental evidence as proof of the view that all plants contain a peroxidase,

* Moore and Whitley, "The Properties and Classification of the Oxidising Enzymes," 'Biochem. Journ.,' vol. 4, 1909.

but only those give a direct action of which the tissues contain more or less organic peroxide.

The experiments I have made with oxidising enzymes corroborate these doubts. I have found that the power to give the direct guaiacum action in any plant is always accompanied by another phenomenon, *i.e.* the formation of brown or reddish-brown pigment when the tissues are injured mechanically or are subjected to chloroform vapour.

Both phenomena are peculiar to certain genera, other genera giving the indirect action only and being unaffected in the same way by injury or by exposure to chloroform vapour. On the whole the direct action is especially characteristic of the Compositæ, Umbelliferae, Labiatae and Boraginaceæ, and certain genera of the Scrophulariaceæ, Rosaceæ, Leguminosæ, Ranunculaceæ, and of many other natural orders. It is absent from or rare in the Cruciferae, Caryophyllaceæ, Crassulaceæ, and Ericaceæ.* The direct action is also more frequent among the Dicotyledons than the Monocotyledons.

The results of my observations have led to the conclusion that the direct action given by the extracts of the plants I have examined† is due to the presence of the dihydric phenol, pyrocatechin, in the tissues of the plants.

That the darkening of plant juices is due to the presence of pyrocatechin has been previously suggested by Grafe.‡ The same suggestion has also been made by Weevers§ in connection with his work on the relationship between pyrocatechin and salicin in *Salix* and *Populus*.

Pyrocatechin rapidly oxidises on exposure to air, and then acts as an organic peroxide, enabling the peroxidase, which is almost universally present, to transfer oxygen to the guaiacum. The plants, such as I have examined, from which pyrocatechin is absent do not give the direct action.

* A similar distribution of direct action has been noted by Passerini, "Sulla presenza di fermenti zimici ossidanti nelle piante fanerogame," 'Nuov. Giorn. Botan. Ital.', 1899. Also by Clark, "The Plant Oxidases," 'Dissertation,' New York, 1910.

† The plants examined included *Aconitum Napellus*, *Caltha palustris*, *Helleborus fatidus*, *Mahonia aquifolium*, *Chelidonium majus*, *Prunus Laurocerasus*, *Pyrus japonica*, *Cornus mas*, *Cheerophyllum sylvestre*, *Ligustrum vulgare*, *Anchusa officinalis*, *Myosotis dissitiflora*, *Rosmarinus officinalis*, *Viburnum Opulus*, *V. Tinus*, *Sambucus nigra*, and *Taraxacum officinale*, in all of which pyrocatechin is present. Pyrocatechin was not detected in *Crocus vernus*, *Galanthus nivalis*, *Arabis albida*, *Eranthis hyemalis*, *Cheiranthus Cheiri*, *Brassica oleracea*, *Viola odorata*, *Primula acaulis*, *Iris germanica*, *Lupinus sp.*, *Narcissus Pseudo-narcissus*, nor in *Arum maculatum*.

‡ Grafe, "Über die Dunkelfärbung von Rübensäften," 'Oest. Zeitsch. f. Zuckerindus. u. Landwirtschaft,' 1908.

§ Weevers, "Die physiologische Bedeutung einiger Glykoside." Extrait du 'Recueil des Travaux Botaniques Néerlandais,' vol. 7, 1910.

These conclusions are based upon three observations:—

(1) Pyrocatechin can be detected by the green reaction with ferric chloride (subsequently purple and red on addition of dilute sodium carbonate) in extracts from plants giving both the direct action and a brown pigment on exposure to chloroform vapour. The alcoholic extract of the plants is evaporated to dryness, and the pyrocatechin extracted with ether or acetone after the removal of chlorophyll and other substances soluble in chloroform. Pyrocatechin was not detected in any appreciable quantity in plants giving the indirect action only.

(2) After evaporation, the ether extract containing pyrocatechin will bring about in many cases a direct blueing of guaiacum when added to a solution containing peroxidase only.* Care must be taken to neutralise the residue (if acid) after evaporation of the ether and before addition of the peroxidase and guaiacum.

(3) When a slightly alkaline solution of commercial pyrocatechin is allowed to stand in air, oxidation takes place and a brown colour is developed. Such a solution added to a peroxidase solution and guaiacum tincture brings about a blueing of the guaiacum. Similar experiments were made with phenol, resorcinol, hydroquinone, pyrogallol, and phloroglucin; also with benzoic, salicylic, *m*-oxybenzoic, *p*-oxybenzoic, protocatechuic, gallic, and tannic acids, and quercetin.

A positive result was obtained with protocatechuic acid only. There is therefore probably a connection between the ortho-position of the hydroxyl groups and the specific capacity of these substances as regards their power to activate the peroxidase. According to Czapek,† protocatechuic acid rarely occurs free in the plant, but further experimental investigation would be necessary to establish this point.

Hence we may conclude that the direct action of certain plant extracts is due to the *post-mortem* oxidation of a definite metabolic product, and the action as such has probably no significance in the metabolism of the living plant.

There is some evidence in favour of the supposition that the pyrocatechin exists as a glucoside, and that the hydrolysis of this compound into sugar and phenol is accelerated by injury or chloroform vapour. In many cases very little oxidation takes place in the alcoholic residue obtained by plunging the leaves of a pyrocatechin-containing plant into boiling alcohol and thereby preventing decomposition of the glucoside. If, however, such a residue is

* Extract of white Brompton Stock was used for this purpose. The guaiacum tincture was always boiled with animal charcoal before using, as recommended by Moore and Whitley, *loc. cit.*

† Czapek, 'Biochemie der Pflanzen.'

boiled with dilute acid in order to hydrolyse the glucoside, considerable oxidation, accompanied by brown coloration, will take place.

Palladin* maintains that the formation of *post-mortem* pigments from aromatic chromogens is proof of the significance of the latter in respiration. Some of the plants from which he obtained the greatest quantity of pigment by treatment of the extracts with peroxidase and hydroxyl are of the pyrocatechin-containing type, and the presence of this phenol, when it occurs, would doubtless accelerate the oxidation of the extracts. But the reactions obtained after death may be no real guide to knowledge of the true metabolic reactions of the living tissues.

The formation of brown pigment on autolysis and injury in pyrocatechin-containing plants is no doubt largely due to the oxidation of the phenol itself, but, in addition, coloration may be caused by the oxidation of other aromatic compounds, *i.e.* tannins, flavones, etc., when once the system peroxide-peroxidase has been established.

The Action of Radium Radiations upon Some of the Main Constituents of Normal Blood.

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(Communicated by Dr. J. R. Bradford, Sec. R.S. Received May 1,—Read June 1, 1911.)

The following experiments were undertaken with a view to determining the effect *in vitro* of the different radiations from radioactive substances upon some of the main constituents of normal blood. The observations have so far been extended to the hæmolytic action of the α -rays on red corpuscles, to the effect of these rays on leucocytes, and to their action on opsonin and complement. Numerous experiments have also been made with the β - and γ -rays, but, generally speaking, the results have been of a negative character.

The Hæmolytic Action of the Emanation.

When radium emanation is mixed with citrated human blood, hæmolysis results. The liberation of hæmoglobin is a gradual process, as is evidenced

* Palladin, "Über das Wesen der Pflanzenatmung," 'Biochem. Zeitsch.,' 1909.